Increased leptin mRNA expression in the blood of dogs naturally infected by *Leishmania infantum*

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**ABSTRACT**

Canine leishmaniosis (CL) is a severe and potentially fatal zoonosis caused by the protozoan *Leishmania infantum*. Severe forms of CL are commonly associated with a non-protective, humoral immune response and high parasitic loads. Leptin, a 16 kD hormone mainly secreted by adipocytes, regulates both the innate and adaptive immunity. The goal of this study was to evaluate leptin mRNA expression levels in blood samples from privately owned dogs with CL (n = 11) and healthy controls (n = 10) using quantitative, real-time polymerase chain reaction. Blood samples from dogs with CL expressed significantly higher leptin mRNA levels (two-fold) compared to healthy controls (P = 0.018). The results suggest a possible involvement of leptin in the pathophysiology of *Leishmania* infection in dogs and the possible use of leptin as a biomarker for CL. Future studies investigating the immunological role of leptin in dogs with CL are warranted.

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RNA was extracted from fresh blood and converted to complementary DNA (cDNA) by reverse transcription. Specific primers from canine leptin and canine glyceraldehyde-3-phosphate dehydrogenase-like (GAPDH) were designed from published mRNA GenBank gene sequences (Table 1). PCR reactions were performed as previously described (Squillaci et al., 2011). All variables, clinical, haematological, and ΔCt (Ctleptin – CGAPDH) values, were subjected to normality testing. Student’s unpaired t test and the Mann–Whitney test were used and P \leq 0.05 was considered significant. Statistical analysis was conducted using MedCalc 12.0 statistical software.

Dogs with CL included seven males (six intact) and four females (three intact), while healthy controls dogs (CN) included five males (all intact) and five females (all intact). The mean age (±standard deviation) at the point of enrolment was 6.36 ± 1.69 years and 5 ± 2.41 years for the CL and CN groups, respectively. The median BCS was 4/9 (range, 4–6) and 5/9 (range, 4–6) for the CL and CN groups, respectively. There was no difference in bodyweight (P = 0.93), age (P = 0.24), and sex (P = 0.55) distributions between the two groups. The more frequent clinical signs observed in CL were lymphadenopathy (72.7%), ocular disorders (45%), splenomegaly (45.5%), moderate to severe generalized exfoliative dermatitis (45.5%) and onychogryphosis (36.4%). The results of haematological parameters are summarized in Table 2. A significant increase in leptin mRNA levels (two-fold) was detected in the CL compared with the CN group (P = 0.018) (Fig. 1).

Our findings show that an increase in leptin mRNA expression levels is present in dogs with CL. There were no differences in bodyweight and nutritional status between the two groups, ruling out obesity as a confounding factor and confirming the possible involvement of leptin in the pathogenesis of protozoan diseases (Baltaci and Mogulkoc, 2012). In addition, although our study evaluated mRNA expression levels in leucocytes and not leptin circulating protein levels, the results could explain the systemic inflammatory status present in CL (La Cava and Matarese, 2004; La Cava, 2012).

Leptin is highly involved in the regulation of the immune system: it stimulates the production of proinflammatory cytokines such as interleukin (IL)-1 and IL-6, it is stimulated by these same cytokines, and it inhibits Tregs (La Cava and Matarese, 2004; Procaccini et al., 2014). These immunological effects may be relevant in severe forms of CL in which alterations of the immune response (reduced circulating and increased tissue Tregs) (Adalid-Peralta et al., 2011; Cortese et al., 2013) and subsequent persistent chronic inflammation could be the effect of a systemic increase in leptin expression.

Because circulating leucocytes include both monocytes and lymphocytes, it is difficult to discern exactly which cell source may be responsible for this increase in leptin mRNA expression. Leptin increased mRNA levels in circulating mononuclear cells could be due to either an increase in total mononuclear cells (increase in mRNA levels compared to controls) and/or to an increased leptin gene transcription in Tregs. This increased transcription in turn may lead to an increase of Tregs in peripheral tissues with lesions, resulting in higher tissue production of leptin reflecting the CL immune dysregulation in tissues.

In conclusion, although the results of this study are preliminary and limited to leptin mRNA expression in circulating mononuclear cells, they suggest an involvement of leptin in CL. Future studies investigating circulating leptin protein levels, the immunological role of leptin in dogs with CL, and the potential utility of leptin as a biomarker of the severity of CL are warranted.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References


